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Function of Tetrapyrroles, Regulation of Tetrapyrrole Metabolism and Methods for Analyses of Tetrapyrroles

Hagen Schlicke[a], Andreas Richter[a], Maxi Rothbart[a], Pawel Brzezowski[a], Boris Hedtke[a], Bernhard Grimm[a]*

[a]*Humboldt-Universität zu Berlin, Lebenswissenschaftliche Fakultät, Institut für Biologie/Pflanzenphysiologie Philippstraße 13, Haus 12, D 10115 Berlin, Germany*

Abstract

Tetrapyrroles are essential cofactors and pigments. Photosynthetic organisms share the use of the highest diversity of tetrapyrrole end products. This report intends to call readers' attention to the recent achievements in research on tetrapyrrole biosynthesis and the current hot topics in tetrapyrrole biosynthesis, including posttranslational control mechanisms and intracellular signaling between the nucleus and the two organelles, plastids and mitochondria. Moreover, the functions of tetrapyrroles in the photosynthetic organisms and valuable information about the current analytical techniques to determine steady state levels of tetrapyrrole intermediates from cyanobacteria, green algae and plant will be surveyed.

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Keywords: Biotechnological application; chlorophyll biosynthesis; gene expression; photodynamic herbicides; postranslational control; transgenic plants.

*Corresponding author. Tel.:+49 302 093 6119; fax: +49 302 093-6337.
E-mail address: bernhard.grimm@rz.hu-berlin.de

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1. Introduction

Tetrapyrroles are probably one of the most ancient prosthetic groups in all organisms and comprise the most abundant pigment molecules on earth. Among the plant tetrapyrroles, chlorophyll and siroheme act in plastids, heme is universally distributed to all cellular compartments and phytochromobiline is assembled into the family of the red/far-red photoreceptor phytochrome in the cytoplasm. Chlorophyll a and b are the most abundant tetrapyrroles on earth. Anaerobic photosynthetic microorganisms synthesize different forms of bacteriochlorophyll. Cyanobacteria and red algae additionally use different phycobilines for light harvesting. During photosynthesis, the Mg^{2+}-chelating (bacterio)chlorophylls are responsible for light absorption and transfer of excitation energy in light-harvesting antenna proteins as well as finally also for the photo-oxidative charge separation in the reaction center of the two protein complexes of photosystem I and II established in the aerobic photosynthetic organisms or in one photosystem of anaerobically grown microorganisms, which developed either the quinone-type or Fe-S-cluster-type photosystem. The porphyrinsheme and siroheme are cofactors of proteins in different cellular processes including respiration (cytochromes), cellular detoxification (e.g., catalase, peroxidase and other heme-dependent proteins) and assimilation of inorganic nitrogen and sulfur from the environment (siroheme in nitrite reductase and sulfite reductase). Heme serves also as sensor for diatomic gases such as O_2, CO and NO and for CO_2 in signal transduction pathways. The phytochromobiline is synthesized from heme and acts as chromophore of phytochromes in light-induced transcriptional control of genes in the nucleus. In contrast to the cyclic tetrapyrroles chlorophylls, heme and siroheme, bilins are linear tetrapyrroles.

The tetrapyrrole biosynthetic pathway is branched leading to the different endproducts. Multiple reviews report about the metabolic pathway with each enzymatic step and the corresponding enzymes[1–4]. While the biochemistry of the enzymatic pathway is well understood and almost all genes encoding enzymes of tetrapyrrole biosynthesis have been identified in plants (Table 1), the posttranslational control and organization of the pathway remains to be clarified and is currently under intensive investigations. In compliance with the specific needs of each cell, the appropriate distribution of tetrapyrroles is accomplished by a network of processes including the synthesis and degradation of tetrapyrroles, their membrane transport, translocation to different cellular sub-compartments and assembly with different apoproteins to functional tetrapyrrole-protein complexes. It is proposed that the coordination of the tetrapyrrole metabolism involves the positioning, assembly and activation of specific sets of enzymes in different organellar subcompartments to balance the supply of intermediates for the synthesis of the different metabolic end products.

As result of the demands for the essential tetrapyrrolic metabolites, the metabolic pathway is controlled at multiples levels of expression. Pathway control begins with transcriptional regulation and ends with different posttranslational mechanisms, including protein degradation. Genes encoding rate-limiting as well as highly regulated enzymes and several regulatory factors of tetrapyrrole biosynthesis seem to be co-expressed by a mainly still unknown set of transcription factors and are tightly controlled by environmental changes (photo period, light intensity, temperature), tissue specificity and endogenous effectors (hormones, e.g. cytokinin, endogenous clock). While transcriptional control causes a long-term and often a daily cyclic modification of the content and, consequently, activity of the nuclear-encoded tetrapyrrole biosynthesis enzymes, posttranslational modification of these proteins serves in the pathway for short term, rapid and transient changes of activity to adjust rapidly the catalytic status of proteins and their activities under fluctuating environmental conditions, such as the varying light intensities.

The most dominant posttranslational control mechanisms have been already described in tetrapyrrole biosynthesis[5]. A redox control by the NADPH-dependent thioredoxin reductase C (NTRC) is exerted on GluTR, CHLM, and POR (abbreviations are explained in Table 1)[6]. Thioredoxin-dependent reduction of CHLI, GSAT, ALAD, UROD and PPOX was hypothesized as result of thioredoxin-dependent proteomic approaches[7]. The Mg chelatase subunit CHLI is reduced by thioredoxin resulting in enhanced ATPase activity and an overall elevated Mg chelatase activity[8].

Additional regulatory factor have been reported: The GUN4 protein binds protoporphyrin and Mg protoporphyrin and activates Mg chelatase by an unknown mechanism[9]. The FLU protein (fluorescent) acts as a negative regulator of ALA-Synthesis in darkness by binding and inactivation of GluTR[10]. A GluTR-binding protein binds a position of GluTR at the membrane and ensures ALA-synthesis also during night when most GluTR are inactivated by FLU[11]. Additional reviews on posttranslational control in tetrapyrrole biosynthesis are finally recommended[5,7,12].

Table 1.Enzymes and regulatory factors in tetrapyrrole biosynthesis of chlorophyll-containing photosynthesising organisms *(selected gene aliases are indicated in brackets).*

Enzymes	Cyanobacteria (*Synechococcus* sp.)	Green alga (*C. reinhardtii*)	Plant (*A. thaliana*)
Glutamyl-tRNAsynthetase (GluRS)	*GltX*	*GTS1* *GTS2*	*ERS*
Glutamyl-tRNA reductase (GluTR)	*HemA*	*GTR (HEMA)*	*HEMA1* *HEMA2* *HEMA3*
Glutamate 1-semialdehydeaminotransferase (GSAT) (Glutamate 1-semialdehyde aminomutase)	*HemL*	*GSAT (GSA)*	*GSA1* *GSA2*
5-Aminolaevulinate dehydratase (Porphobilinogen synthase) (ALAD)	*HemB*	*PBGS (ALAD)*	*HEMB1* *HEMB2*
Porphobilinogen deaminase (Hydroxymethylbilane synthase)	*HemC*	*HMBS (PBGD)*	*HEMC*
Uroporphyrinogen III-synthase (UROS)	*HemD*	*UROS*	*HEMD*
Uroporphyrinogen decarboxylase (UROD)	*HemE*	*UROD1* *UROD2* *UROD3*	*HEME1/UROD* *HEME2*
Coproporphyrinogen III oxidase (anaerobic)	*HemN*	-	AT5G63290
(oxygen dependent) (CPX)	*HemF*	*CPX1* *CPX2*	*LIN2/CPX*
Protoporphyrinogen oxidase	*slr1790/HemJ*	*PPX (PPO)*	*PPOXI, PPOXII*
Ferrochelatase (PPOX)	*HemH*	*HEM15*	*FC1* *FC2*
Mg-protoporphyrin IX chelatase	*ChlD* *ChlI* *ChlH*	*CHLD* *CHLI1* *CHLI2* *CHLH1* *CHLH2*	*CHLD* *CHLI1* *CHLI2* *CHLH (GUN5)*
Mg-protoporphyrin IX Methyltransferase (CHLM)	*ChlM*	*CHLM*	*CHLM*
Mg-protoporphyrin IX monomethylester cyclase (anaerobic)	*ChlE*[a]	-	-
1.1.1. (oxygen dependent)	*1.1.2. sll1214(ycf59)/sll1874* *YCF54*	*CHL27A (CRD1)* *CHL27B (CTH1)*	*CHL27* *LccA*
Divinyl reductase	*slr1923*	*DVR1*	*DVR* (*PCB2*[b])
Protochlorophyllide oxidoreductase (light-dependent)	*POR*	*POR*	*PORA* *PORB* *PORC*
Protochlorophyllide oxidoreductase (light-independent)	*ChlB* *ChlL* *ChlN*	*CHLB* *CHLL* *CHLN*	-
Chlorophyll (bacteriochlorophyll) synthase	*ChlG*	*CHS (CHLG)*	*G4 (CHLG)*
Chlorophyll(ide) a oxygenase	*CAO*	*CAO*[c]	*CAO (CH1)*
Geranylgeranyl reductase	*ChlP*	*GGR*	*CHLP*
Regulators/Chaperones/Assembly factors			
GUN4	*Gun4*	*GUN4*	*GUN4*
Fluorescent		*FLP*	*FLU*
Glutamyl-tRNA binding protein		*GluTRBP*	*GBP*

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Table 1. Continued

Enzymes	Cyanobacteria (*Synechococcus* sp.)	Green alga (*C. reinhardtii*)	Plant (*A. thaliana*)
Light-harvesting-complex like protein 3			*LIL3*
One-helix-protein	*HLIP*	*OHP1*	*OHP1/2*
Tetratricorepeat-peptide proteins	*PITT (TPR)*		*TPR1 and 3*
Early-light-inducible proteins			*ELIP1/2*

[a] found in *Synechocystis* sp. and contains a bchE domain)
[b] *8-vinyl reductase is also annotated as pale-green and chlorophyll B reduced 2(PCB2*
[c] *CAO (only found in Protochlorothrix and Prochloron)*
*Modified and extended from reference[13]

The light-harvesting like proteins (LIL) belonging to the large family of the light-harvesting proteins have been reported to be involved in the cooperation between chlorophyll biosynthesis and the assembly and integration of chlorophyll-binding proteins of the photosynthetic machinery[14]. Depending on the number of LHC-like transmembrane domains, it is distinguished between one helix proteins (OHP, HLIP High-light-inducible protein), two-helixproteins (SEP1 and 2, stress-enhanced protein 1 and 2, LIL3) and three-helix-proteins (ELIPs, early light inducible proteins).These proteins are reported to be involved instress-related modulation of chlorophyll accumulation, assembly of chlorophyll-binding proteins as well as stability and turnover of components of the two photosystems[15,16]. LIL3 stabilizes the geranyl-geranyl-reductase (GGR) in the thylakoid membrane[17]. But in most cases the physical interaction between these LIL proteins and the proteins of chlorophyll metabolism as well as the assembly of photosynthetic protein complexes awaits still elucidation.

The metabolic pathway control of tetrapyrrole biosynthesis includes also intracellular exchange of information between organelles and the nucleus[18,19]. This concept of intracellular communication comprises an anterograde and retrograde signalling network. While the organellar gene expression is controlled by the genetic information of the nucleus, the nuclear transcriptional control is also affected by metabolic and genetic activities of the organelles, plastids and mitochondria, to adjust the protein synthesis to the requirements in organelles in response to development and abiotic stress. It has been reported that modification of tetrapyrrole biosynthesis also modulates expression of genes encoding plastid-localized proteins. While the concept of tetrapyrrole-dependent retrograde signalling is generally accepted, it is not clear what the emitters of these signals are and how the signal is communicated towards cytoplasm and nucleus (see also Schlicke et al.[20]).

Ultimately, any modification in the metabolic pathway of tetrapyrrole biosynthesis affects the steady state levels of intermediates. Conversely, analyses of the steady states level of metabolites is an excellent measure for the biochemical state of plants under different adverse condition or for a mutant screen in comparison to control plants. It is a challenging task to assay the minute amounts of metabolites to assess the metabolic activities of the tetrapyrrole pathway. A series of application to qualitatively and quantitatively determine tetrapyrrole intermediates in model organisms, such as Arabidopsis and tobacco (see most recent reviews on methods in tetrapyrrole analysis[21,22]. These reports describe the procedures from extraction up to evaluation of quantitative data. Moreover, they include also methods for quantification of non-covalently bound heme or enzyme activities in this pathway. Use of mass spectrometry to determine Mg porphyrins are reported in[23]. All methods based on the spectral properties of chlorophyll and its tetrapyrrole precursors. While spectroscopy provides a fast assessment of the pigment quantity it has to be taken into account that the determined spectrum is the sum of all solubilized pigments. High performance liquid chromatography (HPLC) enables separation of pigments according to their biochemical properties and subsequent detection by absorbance or fluorescence using a photodiode array and fluorescence spectrometer, respectively.

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